Abstract

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LKB1/AMPK is the main cellular energetic pathway, which acts as the sensor of various extra- and intracellular signals. In response to the signals, it regulates energy metabolism and the maintenance of cellular homeostasis, so its major role lies in the survival, growth and development of the whole organism. This pathway is of significant importance because it has the potential to suppress tumoral progress in the cell. Metformin, the widely used drug in the treatment of type 2 diabetes, exerts a significant antitumour activity. However, the direct mechanism of metformin´s action is still unknown. Metformin induces cellular stress similar to the metabolic stress via inhibiting mitochondrial respiratory chain complex I, which results in an increase of AMP levels in plasma. AMP then binds to the AMPKγ subunit, so metformin mediates the activation of AMPK. AMPK is suddenly phosphorylated on Thr^{172} of the AMPKα kinase domain via LKB1, which mediates the downregulation of many downstream kinases. The result is the regulation of the metabolism on the gene expression level. Catabolic processes (glycolysis, fatty acid oxidation) and autophagy are induced, and anabolic processes are inhibited. Metformin also reduces the expression of the main biotransformation genes of cytochrome P450 (CYP3A4, CYP2C9 and CYP2B6) via inhibition of the PXR nuclear receptor. Metformin disrupts the interaction of PXR with his coactivator SRC1, and impairs the function of PXR. In this study we have introduced the immunodetection method of Western blotting. Using specific antibodies, we detected the AMPKα phosphorylated form in the LS174T cell line derived from the human colon adenocarcinoma. The presence of the phosphorylated form correlates with the activation or inhibition of this kinase. Through the method of Western blotting we established that the model activators of AMPK, metformin and AICAR, increased the density of the bands for the phosphorylated form of AMPK, but did not increase for nonphosphorylated forms AMPKα and $AMPK_{\beta 1/2}$. This gives evidence of the activation of the AMPK pathway in the LS174T cell line. Furthermore, the inhibitor of AMPK, the compound BML275, decreased the density of the band of the phosphorylated AMPKα. Thus, after further optimizing this method (e.g. optimizing titration concentrations and antibody dilutions, optimizing the optimal time of incubation with antibodies and the washing time) it will be possible to use this method for research purposes. We believe our analysis will contribute to the search for the metformin mechanism of action and will also contribute to the explanation of its effect on the expression of the biotransformation enzymes.